Journal of Research on the Lepidoptera 7 (1): 23-28, 1968

1160 W. Orange Grove Ave., Arcadia, California, U.S.A. 91006

METHODS FOR STUDYING THE CHROMOSOMES OF LEPIDOPTERA

THOMAS C. EMMEL

Department of Zoology, University of Florida, Gainesville 32601

Chromosome information has been published on over two hundred butterfly species in the Palearctic region (de Lesse, 1960, and included references). A smattering of chromosome counts has been published on African species of Rhopalocera (de Lesse and Condamin, 1965, 1966), and an initial survey of 105 Nearctic and northern Neotropical species has been given by Maeki and Remington (1959, 1960a, 1960b, 1960c). Some Australian species have been studied cytologically (Emmel & McFarland, unpublished). Recently, de Lesse (1967) published a list of the chromosome numbers of 284 Neotropical Rhopalocera, all from South America; these include 14 species already counted by Maeki and Remington from northern Mexico. These references are the only major publications to date on butterfly chromosomes. Yet with the exception of Drosophilidae no other large group of animals approaches the degree of cytotaxonomic knowledge (over 700 species) we now have of the Rhopalocera (Maeki and Remington, 1960c). The karyotypes of the moths are almost totally unknown.

In the course of extensive investigation of the karyotypes of Neotropical and Nearctic butterfly species, the author has developed a simplified set of techniques for obtaining and studying the chromosomes of Lepidoptera. The purpose of the present paper is to outline these methods.

COLLECTION AND PRESERVATION OF TESTES

Chromosomes are most easily studied in dividing cells in the testes of male butterflies. Meiosis usually continues there for some time after eclosion (up to several months in *Heliconius* species), the haploid number is easier to observe than the diploid complement of mitotic somatic cells, and meiosis in the eggs of a female only occurs singly during the short interval of sperm combination with each egg (see also Maeki and Remington, 1959).

In almost all butterflies, the two testes are fused laterally and located at the *top* of the abdomen, beneath the junction of the third and fourth abdominal segments counting forward from the genitalia (the easiest way to count in the field). In the large sulfur genus *Phoebis* and certain other Neotropical butterflies, the testes are placed in the top of the clasping apparatus (terminal abdominal segment).

Location: The roundish or oblong testes are always in the *center* and have two long tubes attached to their joined base.

Color: They are usually *rose* or *red*, but may be *greenish, black, yellow*, or even *clear* in many lycaenids, satyrids, and certain Papilios.

Size: This varies greatly, depending on age (decreasing size in older individuals) and the species. *Heliconius* and danaids have testes up to 2 mm in diameter. In some satyrids and lycaenids (blues), they may be only 0.1-0.2 mm in diameter.

Directions for Removal of Testes:

To remove the testes, hold the male butterfly in the left hand with its wings above the thorax; shove the abdomen at an angle downwards with a free finger. With fine watchmaker's forceps (No. 5 size is best) in the right hand, tear open a slit in the top of the abdomen at the junction of the third and fourth segments back from the claspers (Fig. 1). The distinctively-colored testes should pop into view immediately; the other abdominal contents are yellowish, white or translucent.

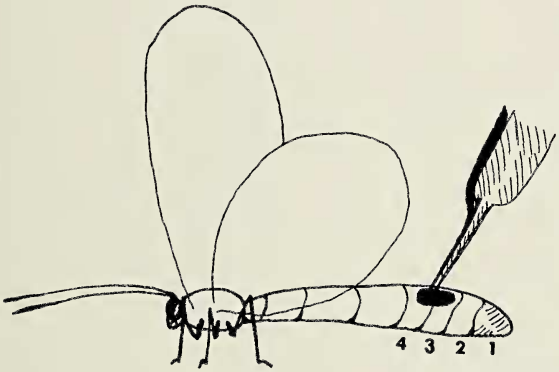
Pull off the testes and place into a screw-cap vial (one- or two-dram size are satisfactory) containing a 3:1 mixture by volume of absolute ethyl alcohol and glacial acetic acid (freshly mixed or not more than a few hours old). Push the abdominal contents back in the male's abdomen, close the slit, and store the specimen in an envelope for future reference. Put a label with a pencil-written code number in the vial with the testes; put the same number in ink on the glassine envelope containing that male specimen; and enter the same number and collection data in a permanent notebook. The wing condition or apparent age of the male can be noted, also. Use a different vial for the testes of each individual butterfly.

The testes can be stored in the original vials until chromosome squashes are made; it is not necessary to transfer them to alcohol for storage. The vials should be refrigerated and if possible

stored at freezing temperatures at the earliest opportunity. The testes will give satisfactory chromosome perparations even after two years of frozen storage, though faster processing is recommended.

Because some individuals in a population may not be undergoing active spermatogenesis at the same time as others, it is advisable to collect 5-10 testes for each species (or more if there is known to be a variable number of chromosomes in populations of that species).

The collection of testes from live males does not have to be done immediately upon netting of the specimens. Males can be left alive (unpinched) in glassine envelopes all day as long as they do not get overheated, and the testes can be removed in the evening as each male is killed.



1

SQUASH TECHNIQUE FOR CHROMOSOME STUDIES

The following squash technique is the simplest and fastest one to use to obtain good preparations of chromosomes which then may be photographed and drawn via camera lucida for permanent record. This procedure does not produce permanent slides, though these squash preparations may be sealed with clear nail polish and held at freezing temperatures for several years if desired. However, slides may be made permanent by dehydrating the preparations in a series of alcohol concentrations (Guthrie, Dollinger, & Stetson, 1965).

1. After removing testes from fixative in the vial or from a freshly-killed male, place on a clean slide in a drop of Lacto Orcein Stain (see Appendix I).
2. Macerate the testes with watchmaker's forceps. Allow to

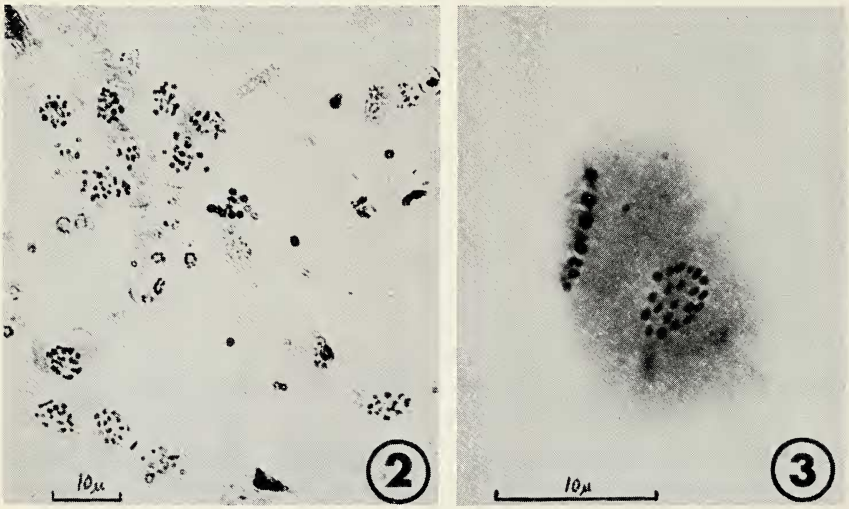


Fig. 2. General field of dividing testicular cells at 400x microscope magnification. *Eurema* sp. (Pieridae), San Vito de Java, Puntarenas Province, Costa Rica (n=29). It is relatively rare to obtain cells with all chromosomes in the same plane of focus; only one or two of the sets of chromosomes in this field of view could probably be counted accurately (at higher magnifications).

Fig. 3. Highly magnified haploid set of chromosomes (n=21) from a testis cell of a *Heliconius melpomene* male (Nymphalidae: Heliconiinae), Osa Peninsula, Puntarenas Province, Costa Rica. The line of chromosomes at left represents an equatorial view of chromosomes on a metaphase spindle in a neighboring cell. The microscope magnification for this photograph was 1000x.

stand for at least 5 minutes. (A longer exposure to the stain, up to several hours, gives better results. Cover stain drop with a small watch glass to prevent evaporation).

3. Place cover slip over drop of stain. Tap the top of the cover slip to spread out cells.

4. Put a paper towel or filter paper on top of cover slip and squash by thumb pressure over cover slip. (Or, the slide may be inverted, placed on a sheet of glass with paper toweling above and below, and thumb pressure applied to squash.) A Carver Laboratory Press may be used to insure a uniform, well spread chromosome preparation.

5. Remove excess stain around edges of cover slip with filter paper and examine slide under low power (100x) to locate areas of dividing cells.

Dividing cells may be examined under oil-immersion for counting, description and photography. The author uses a Carl Zeiss Research Microscope STANDARD WL fitted with plan-apochromatic flat-field objectives and automatic camera. An oil-immersion Planapo 100x objective is used for critical observation and photography. Total magnifications of at least 1000x are needed for studying the tiny chromosomes of the Lepidoptera.

Examples of the appearance of areas of dividing testicular cells and of chromosomes at high magnification are given in Figures 2 and 3.

ACKNOWLEDGMENTS

This research has been supported by the N.I.H. Genetics Training Grant to the University of Texas at Austin (1968-1969) and by N.S.F. Grant GB8442. I thank Dr. Guy L. Bush for his great assistance at the University of Texas in the initiation of this chromosome research on Lepidoptera and for his continued interest and support.

LITERATURE CITED

DE LESSE, H. 1960. Speciation et Variation chromosomique chez les Lepidopteres Rhopaloceres. *Annales des Sciences Naturelles, Zoologie,* 12e Serie, 2(1): 1-123.

DE LESSE, H. 1967. Les Nombres des Chromosomes chez le Lepidopteres Rhopaloceres Neotropicaux. *Ann. Soc. ent. France* (N.S.), 3(I): 67-136.

DE LESSE, H., and M. CONDAMIN. 1965. Formules chromosomiques de quelques Lepidopteres Rhopaloceres du Senegal et de Cote d'Ivoire. *Bulletin I.F.A.N.,* 27, A(3): 1089-1094.

DE LESSE, H., and M. CONDAMIN. 1966. Formules chromosomiques de quelques Lepidopteres Rhopaloceres d'Afrique Centrale. *Ann. Soc. ent. France* (N.S.), 11(2): 349-353.

GUTHRIE, W. D., E. J. DOLLIINGER, and J. F. STETSON. 1965. Chromosome studies of the European corn borer, Smartweed borer, and Lotus borer (Pyralidae). *Annals Ent. Soc. Amer.,* 58(1): 100-105.

MAEKI, KODO, and CHARLES L. REMINGTON. 1959. Studies of the chromosomes of the North American Rhopalocera. 1. Papilionidae. *Journ. Lepid. Soc.,* 13: 193-203.

————. 1960a. *Idem.* 2. Hesperiidae, Megathymidae, and Pieridae. *Journ. Lepid. Soc.* 14: 37-57.

————. 1960b. *Idem.* 3. Lycaenidae, Danaeinae, Satyrinae, Morphinae. *Journ. Lep. Soc.,* 14: 127-147.

————. 1960c. *Idem.* 4. Nymphalinae, Charaxidinae, Libytheinae. *Journ. Lepid. Soc.,* 14: 179-201.

APPENDIX I

Lacto Orcein Stain

1 gm	Orcein
40 cc	Glacial Acetic Acid
10 cc	Lactic Acid (undiluted)
50 cc	Water (distilled)

Bring to a boil, let mixture stand in flask overnight, then filter with Whatman No. 1 filter paper to remove crystals. Store stain in small nose-dropper bottles.